Miniprep of 4 RFP in PSB1C3 Overnight Cultures Using Qiagen Qiaprep Kit

Made with Benchling

Project: Awesome Possum

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Purpose:

Extract plasmid DNA from overnight cultures so it can be digested and then ligated with LT10 + promoter and LT15 + promoter constructs.

Materials:

- 4x 5.0 mL RFP in PSB1C3 from glycerol stock overnight cultures
  - Labeled: "A" "B" "C" "D"
- Qiagen Qiaprep Kit
- Invitrogen Purelink Spin Columns
- Benchtop Centrifuge (max 4,000 RPM swinging bucket rotor)
- 4x eppendorf tubes
- Micropipette
- Sterile Micropipette tips
- Vortexer
- Implen 300

Protocol:

Overnight cultures were retrieved from the shaker/incubator in the morning. The culture liquid appeared cloudy with white sediment at the bottom of each 15.0 mL screw-top falcon tube. First, all 4 tubes were centrifuged for 15.0 minutes at 4.0˚C 4,000 RPM. Then supernatant was decanted into a waste container (all visible pellets appeared light red-brown in color). Next, 250.0 µL of buffer P1 was added to each tube and then vortexed to resuspend pellets. Then, resuspensions were transferred to individually labeled eppendorf tubes with a micropipette. After that, 250.0 µL of buffer P2 was added to each tube and then inverted several times to mix. Then 350.0 µL of buffer N3 were added to each tube and then inverted several times to mix. Next, all 4 tubes were centrifuged at 25.0˚C for 10.0 minutes 13,000 RPM. After that, a micropipette was used to transfer supernatants into Invitrogen Purelink spin columns which were placed inside 1.5 mL recovery tubes. The column/recovery tubes were then centrifuged for 30.0 seconds at 25.0˚C 13,000 RPM and emptied of flow through. Next, 500.0 µL of buffer PB was added to all spin columns. The columns were then centrifuged for 60.0 seconds at 25.0˚C 13,000 RPM and emptied of flow through. Then, 750.0 µL of buffer PE was added to each spin column with a micropipette. The spin columns were centrifuged for 60.0 seconds at 25.0˚C 13,000 RPM and emptied of flow through. Next, the spin columns were centrifuged at the same settings for an additional 60.0 seconds. Finally, the columns were placed into sterile labeled eppendorf tubes and then eluted with 50.0 µL of buffer EB. The tubes were incubated at room temperature for 60.0 seconds and then centrifuged for 60.0 seconds at 25.0˚C 13,000 RPM.

DNA Concentration Check with Implen P 300

All Mini-Prepped samples were measured with an Implen P 300 nano-photometer for DNA concentrations. The nano-photometer was blanked with 1.0 µL of buffer EB and then 1.0 µL of each construct was measured. Please see table 1 for concentration values.

Table 1: DNA Concentrations of Mini-Prepped RFP in PSB Cultures

	A	B	C	D	E	F	G
1	**Sample #**	**Concentration (ng/µL)**	**A230**	**A260**	**A280**	**A320**	**A260/A280**
2	A	228	0.042	0.091	0.049	0	
3	B	228	0.04	0.091	0.049	0	
4	C	205	0.034	0.082	0.042	-0.001	
5	D	138	0.024	0.055	0.029	-0.001	